The influence of elevated atmospheric CO₂ on fine root dynamics in an intact temperate forest

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Abstract

Root dynamics are important for plant, ecosystem and global carbon cycling. Changes in root dynamics caused by rising atmospheric CO₂ not only have the potential to moderate further CO₂ increases, but will likely affect forest function. We used FACE (Free-Air CO₂ Enrichment) to expose three 30-m diameter plots in a 13-year-old loblolly pine (Pinus taeda) forest to elevated (ambient +200 µL L⁻¹) atmospheric CO₂. Three identical fully instrumented plots were implemented as controls (ambient air only). We quantified root dynamics from October 1998 to October 1999 using minirhizotrons. In spite of 16% greater root lengths and 24% more roots per minirhizotron tube, the effects of elevated atmospheric CO2 on root lengths and numbers were not statistically significant. Similarly, production and mortality were also unaffected by the CO₂ treatment, even though annual root production and mortality were 26% and 46% greater in elevated compared to ambient CO₂ plots. Average diameters of live roots present at the shallowest soil depth were, however, significantly enhanced in CO2-enriched plots. Mortality decreased with increasing soil depth and the slopes of linear regression lines (mortality vs. depth) differed between elevated and ambient CO₂ treatments, reflecting the significant CO₂ by depth interaction. Relative root turnover (root flux/live root pool) was unchanged by exposure to elevated atmospheric CO₂. Results from this study suggest modest, if any, increases in ecosystem-level root productivity in CO₂-enriched environments.

Keywords: elevated CO₂, Pinus taeda, root production, root turnover, root length, FACE Received 19 January 2001; revised version received 3 April 2001 and accepted 29 June 2001

Introduction

Substantial experimental evidence indicates that a doubling of atmospheric CO₂ will increase rates of photosynthesis and biomass accumulation in most plant species. Root systems often realize the most consistent and largest increase in growth (Rogers *et al.* 1994; Batts *et al.* 1998; Pritchard *et al.* 1999), sometimes, but not always, result-

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ing in greater root: shoot ratios (Wullschleger *et al.* 1995; Curtis & Wang 1998). Evaluations of standing root crop biomass and root: shoot ratios, however, may underestimate the total amount of assimilated C transported below-ground. Several studies have shown, for example, that observed increases in canopy C assimilation resulting from growth in high CO₂ could not be accounted for by an increase in above- or below-ground biomass (Fitter *et al.* 1997; Cheng & Johnson 1998).

Fixed C unaccounted for in the living tissues of plants grown under CO₂-enrichment could have a number of

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below-ground fates including: (i) respiration for new growth or maintenance, (ii) diversion to symbionts such as mycorrhizae, (iii) loss through herbivory, (iv) loss as exudates, or shoughed cells, and (v) loss in the process of root turnover. It has been suggested that turnover of finer root elements in forest ecosystems might consume more energy and carbon than maintenance respiration, exudation, C transfer to symbionts and below-ground herbivory (Caldwell 1977; Canadell et al. 1996). But little quantitative information is available concerning the fate of the extra carbon assimilated and translocated belowground in CO2-enriched atmospheres. The objective of this study therefore was to characterize the effects of elevated atmospheric CO₂ on ecosystem root dynamics including root production, mortality, vertical distribution, and turnover.

Because of our limited understanding of the controls on root longevity, it is difficult to speculate on the potential effects of elevated atmospheric CO2 on root dynamics (Pritchard & Rogers 2000). Van Noordwijk et al. 1998) recently presented a quantitative discussion on the potential impact of growth in CO₂-enriched atmospheres on the costs and benefits of maintaining old roots vs. growing new ones. They theorized that increasing atmospheric CO₂ is unlikely to exert a significant direct effect (mediated by greater C supply) on root turnover (but see Marshall & Waring 1985). In contrast, Eissenstat et al. (2000) used an efficiency model to show that reduced tissue N concentrations and reduced root maintenance respiration, both of which are predicted to result from rising CO₂, will more likely result in slightly longer root life spans (reduced root turnover). Existing data concerning the influence of elevated atmospheric CO₂ on root dynamics, especially at the ecosystem level, are few and too highly variable to either support or refute these hypotheses.

We characterized the influence of elevated atmospheric CO₂ concentrations on seasonal growth and turnover of roots in a loblolly pine (Pinus taeda) forest located in the Blackwood Division of the Duke Forest, North Carolina, USA over a period of 1 year. Beginning in August 1996, FACE (Free-Air CO2 Enrichment) technology was used to expose a 13-year-old loblolly pine forest to CO_2 -enriched air (ambient +200 μ L L⁻¹). A significant (50-60%) increase in leaf level photosynthesis and a 25% increase in primary production during the first 2 years of the experiment have been reported (DeLucia et al. 1999). Furthermore, Matamala & Schlesinger 2000) recently characterized root dynamics in this experiment using sequential soil coring. They found an increase in live fine root biomass and a trend towards greater root net primary production (RNPP) in elevated compared to ambient plots after 2 years of exposure. Because of the many difficulties associated

with measuring root production, use of more than one method is often advocated (Matamala & Schlesinger 2000). This report is the first to use minirhizotrons to characterize the effects of CO₂-enriched air on root dynamics within an intact mature forest and is intended to complement root data acquired with sequential soil cores by Matamala & Schlesinger (2000).

Materials and methods

The FACE (Free-Air CO₂ Enrichment) site is located in the Piedmont region of North Carolina, USA, where a loblolly pine forest was planted in 1983 after harvest of similar vegetation (Matamala & Schlesinger 2000). The density of pine trees (1733 stems ha⁻¹) represents more than 98% of the total basal area in this forest. Several deciduous trees have become established including red maple (*Acer rubrum*, 207 stems ha⁻¹), winged elm (*Ulmus alata*, 226 stems ha⁻¹) and sweetgum (*Liquidambar styraciflua*, 620 stems ha⁻¹). The soil series is Enon loam (fine, mixed, thermic Ultic Hapludalfs).

The experiment consists of six experimental rings. Each ring is 30 meters in diameter. Briefly, CO_2 is delivered through a series of perforated vertical pipes that extend to the top of the canopy. Three fully instrumented control plots receive ambient air, and three are enriched with CO_2 (ambient $+200~\mu L~L^{-1}$). Fumigation was initiated on 27 August 1996 and stopped only when temperature dropped to below 5 °C or the wind speed was higher than 5 m s⁻¹ for more than 5 min; plots were fumigated 80% of the time from 1997 to 1998. Details of FACE technology were reported by Hendrey *et al.* (1999).

Minirhizotron analysis of root dynamics

A total of 72 minirhizotrons (12 per ring) were installed between 23-26 June 1998. Minirhizotrons are clear plastic tubes (OD = 56 mm) that allow repeated, non-invasive measurement of root growth. These clear tubes were installed at an angle of 45° from vertical to a vertical depth of 31 cm. FACE rings were divided equally into four sectors; three tubes were installed at random with respect to vegetation into each of the four. The portion of the minirhizotron tube extending above the ground was covered with a closed-cell polyethylene sleeve, and the end was sealed with a rubber cap to exclude light and minimize heat exchange between the air and the tube. A PVC cap was then installed over the end to protect the rubber cap from UV damage, and to further protect and insulate the tube. In order to prevent minirhizotron tubes from moving, customized aluminium brackets (design courtesy of Mark Johnson, EPA, Corvallis) were clamped to tubes and anchored into the ground with 40-cm stainless steel rods. No data were collected for the first four months after tube installation to allow time for roots to colonize the tube surface, and for soil adjacent to minirhizotrons to equilibrate (in terms of bulk density and root length density) with surrounding soil.

From 22 October 1998, A BTC-100x microvideo camera (Bartz Technologies, Santa Barbara, California) was inserted into minirhizotrons approximately every 4 weeks and images of roots growing against the tubes were recorded. The camera was equipped with an indexing handle allowing very precise and consistent camera placement over time (Johnson & Meyer 1998). Video images of roots were recorded 12 times from 22 October 1998 to 23 October 1999. Video frames were replayed in the laboratory, still images were manually digitized, and root data were extracted using the image analysis program RooTracker (Dave Tremmel, Duke University). A total of 16 frames representing depths from 0 cm to 31 cm were analysed from all 72 tubes at each date for a total of 13 824 images (16 depths \times 72 tubes \times 12 dates).

Several variables were recorded for each minirhizotron frame at each date including the number of live roots, number of dead roots, length of live roots, length of dead roots, total diameters of live roots (number of roots visible in the minirhizotron image multiplied by the average diameter per root), and total diameters of dead roots (number of roots that have died within a frame multiplied by their average diameter). Roots were considered dead when they either disappeared from view or when their structural integrity began to deteriorate (e.g. upon root fragmentation). Therefore, mortality includes all roots lost to grazing by soil herbivores. In some cases, roots may indeed have been dead and non-functional, but because they were still visible in minirhizotron video frames, they were still considered live.

From these basic data, root production per day, root mortality per day, and average diameter per root was calculated and the data presented. RNPP is defined as the rate of construction of new roots. Root length measures were converted to masses using methods outlined by Tingey et al. (2000) assuming a 2.5-mm depth of field. Briefly, root length density was calculated by dividing the length of roots observed in each minirhizotron frame by the soil volume observed $(182 \text{ mm}^2 \text{ area} \times 2.5 \text{ mm} \text{ depth of field} = \text{soil volume})$ in mm³). To convert length to biomass, root length density was divided by specific root lengths of 12.17 (ambient CO₂) or 11.14 (elevated CO₂) which were derived from soil cores by Matamala & Schlesinger (2000). Resulting values were converted to g m⁻² by multiplying by the depth of the soil profile sampled.

Root turnover is a term that is often used to describe aspects of root formation, death and decomposition

(Tingey et al. 2000). Root turnover is often used synonymously with root mortality, but such a definition provides little information concerning the flux of roots (mortality) through the total root pool (standing crop). That is, it provides very little information about how rapidly standing root crop biomass disappears. For the purpose of this study, we define relative root turnover as a unitless index relating standing crop to root mortality over monthly intervals. Relative root turnover was calculated by dividing mortality (length) at time x by standing root crop (length) at time x-1.

Experimental design

The experimental design was a split block design with three replications (three elevated rings and three ambient rings). A block consisted of two rings to which ambient and elevated CO2 treatments were assigned. Each experimental ring contained 12 subsample minirhizotron tubes. The minirhizotron frames were grouped into four depth classes in order to determine treatment effects on vertical root distribution (depth 1 = 0-7.7 cm, depth 2 = 7.7-15.5 cm, depth 3 = 15.5-23.1, and depth 4 = 23.1-31 cm). The subunit factor depth is a split block because depths were not randomly assigned within the minirhizotron frames (Steel et al. 1997). Measurements were taken on each tube on 12 dates.

Initial analysis of variance was performed on the data combined over all dates. In this analysis, dates were treated as an additional split block subunit. As date did not interact with CO₂ or depth factors, CO₂ and depth means averaged over all dates are presented. SAS PROC MIXED was used for the analysis of variance (SAS 1999). Because there was a significant CO₂ by depth interaction for several response variables, we used linear regression of least square means to investigate the difference in response to depth for the two CO₂ concentrations. Regression slopes and intercepts were compared using the Student's t-test as outlined by Zar (1996). Differences were considered statistically significant when $P \le 0.10$.

Results

Standing root crop

Total root length decreased significantly with depth (P = 0.002) at all sampling dates (Fig. 1). In general, greater variability in visible root lengths was present at shallow compared to deeper soil depths. Of root length visible in the top 31 cm of the soil profile, 43% was in the first (0-7.7 cm) depth category, 32% in the second (7.7-15.5), 16% in the third (15.5-23.1), and 10% in the fourth (23.1-31 cm). Maximum standing root length was observed in January 1999 for the 0-15.4 cm depth,

compared to August and September 1999 for the 15.4–31 cm depth.

Although elevated CO_2 increased total standing root length by 16%, and root number per frame by 24%, these changes were not significant (Table 1). There was, however, a significant CO_2 -by-depth interaction (P = 0.09) which revealed an increase in root length in the first and third depth classes. But, as seen in Fig. 2A, when modelled with a linear regression, the slopes of regression lines for elevated and ambient CO_2 treatments did not differ.

There was also a significant CO_2 -by-depth interaction for average diameters of live roots (P = 0.09; Fig. 2B). In general, diameters of live roots were greater in the CO_2 enriched plots compared to ambient plots only in the shallowest soil depth class. Regression analysis of this relationship showed that diameters of live roots increased with depth and the slope of the response differed significantly between CO_2 treatments (P < 0.10, Fig. 2B). No consistent effects of the CO_2 treatment were observed for average diameters of roots that died during the course of the experiment (Fig. 2C). The linear

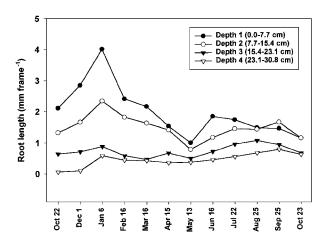


Fig. 1 Temporal and spatial distributions of root length from October 1998 to October 1999. Values are averages of both ambient and elevated CO_2 treatments (all 72 minirhizotron tubes).

relationship of dead root diameter to depth did not differ significantly between CO₂ treatments.

Root dynamics and net primary productivity

Root length production (P = 0.42) and mortality (P = 0.28) per day were +16% and +34% greater in elevated compared to ambient CO_2 conditions (Table 2). Similar to results obtained for standing root length, a significant CO_2 -by-depth interaction for root length mortality per day was observed (P = 0.04; relationship nearly identical to mortality per day expressed as biomass as shown in Fig. 4B). Although non-significant, greater root production per day generally resulted in enhanced yearly cumulative root length growth (Fig. 3). Relative root turnover was not affected by CO_2 concentration (Table 2).

Annual root production was estimated to be 181.1 and 228.6 g dry wt m⁻² year⁻¹ in the ambient and elevated CO_2 plots, respectively (P=0.24; Table 3, Fig. 4A). No significant CO_2 by date or depth interactions were observed (Fig. 4A). Annual root mortality was 34.8 g dry wt m⁻² year⁻¹ in ambient CO_2 plots compared to 50.8 g dry wt m⁻² year⁻¹ in elevated plots (P=0.2006; Table 3). There was a significant CO_2 -by-depth interaction for mortality per day expressed as g dry wt m⁻² day⁻¹ (P=0.03; Fig. 4B). Mortality decreased with increasing soil depth and the slopes of the response variables differed significantly between CO_2 treatments (P<0.10).

Discussion

As previously reported by Matamala & Schlesinger (2000) for this study site, roots were distributed predominantly in the top soil layers with 90% of root length present in the top 23 cm of soil. Maximum root lengths were generally observed in early winter and late summer. There were no significant main effects of elevated CO₂ on rooting. Although not statistically significant, standing root length and root numbers per minirhizotron tube were enhanced by 16% and 24% in CO₂-enriched

Table 1 Main effects of CO₂ level on length of live roots, live root number and average diameter of live and dead roots collected with minirhizotrons from October 1998 to October 1999. Values are means of 3 replicates (each with 12 minirhizotrons) averaged over all depths and dates

	Elevated	Ambient	F value	<i>P</i> > <i>F</i>
Live root length (mm per frame)	1.24	1.07	1.48	0.3478
Live root number (no. per frame)	0.21	0.17	2.23	0.2740
Diameter of live roots (mm)	0.83	0.77	0.72	0.4868
Diameter of dead roots (mm)	0.87	0.82	0.39	0.5980

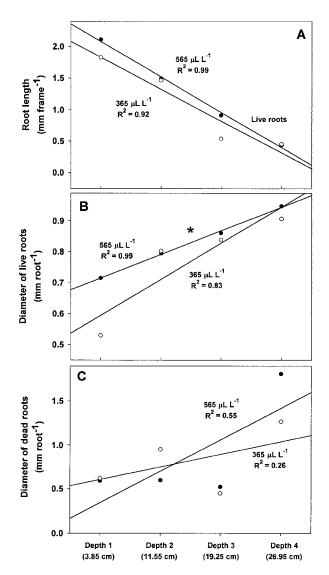


Fig. 2 $\mathrm{CO_2}$ -by-depth interactions for (A) live root length per minirhizotron frame (P=0.09), (B) average diameters of live (P=0.09) and (C) dead roots. Values are averaged over dates. * indicates that the slopes of the regression lines differed at P<0.10.

plots. These increases are considerably smaller than those commonly reported for individually grown forest trees exposed to high CO₂. For example, fine root density increased 135% in *Pinus sylvestris* (Janssens *et al.* 1998) and Crookshanks *et al.* (1998) reported 95–240% increases in fine root production of *Quercus petreae*, *Pinus sylvestris*, and *Fraxinus excelsior*. Our data suggest that these previous studies on tree root response to CO₂ enrichment may have exaggerated the effects of elevated CO₂. Although root biomass is almost always stimulated by CO₂ when plants are grown within growth chambers or glasshouses, root biomass at the ecosystem level appears to increase to a much smaller extent (Pritchard *et al.* 2001).

As pointed out by McLeod & Long (1999), studies of root growth conducted in open top chambers (OTC) may be compromised by artifactual air movement, and thus contribute to overestimates of root responses. In particular, air movements fostered by the open top chamber design might increase the anti-transpirational effect of CO₂, in the process artificially increasing soil moisture. An artifactual effect on soil moisture could be especially important when assessing fine roots (McLeod & Long 1999). However, another recent OTC study conducted on trees growing in the ground, in competition with several other plant species, also reported a smaller effect of elevated CO₂ on rooting than has typically been reported for isolated pot grown trees (Pritchard et al. 2001). Hence, competitive effects, as opposed to effects caused by the experimental apparatus itself, could also be causing such contrasting results.

Relative root turnover (root flux/live root pool) was not significantly changed by CO₂ enrichment in this study, implying no change in root longevity. This result is consistent with earlier reports which also found no change in relative root turnover in this forest (Allen *et al.* 2000; Matamala & Schlesinger 2000). It is important to remember, however, that this study ran for only 1 year, and therefore, few roots actually died (Fig. 3). A mean residence time of 3 years for fine roots was estimated by dividing the mean standing biomass by the annual net production in the ambient treatment (Matamala &

Table 2 Main effects of CO₂ treatment on root length relative turnover, production per day and mortality per day collected with minirhizotrons from October 1998 to October 1999. Values are means of 3 replicates (each with 12 minirhizotrons) averaged over all depths and dates

	Elevated	Ambient	F value	P > F
Production (mm frame ⁻¹ day ⁻¹)	0.0103	0.0089	1.01	0.4210
Mortality (mm frame ⁻¹ day ⁻¹)	0.0023	0.0017	2.11	0.2835
Relative root turnover (mo ⁻¹)*	0.053	0.056	0.02	0.9108

^{*}Root turnover is a relative index of root flux (root mortality time x/standing root length time x-1) calculated over monthly intervals

Schlesinger 2000). If this estimate is correct, 3–4 years of root data will be required before a meaningful cohort analysis, the most direct way to quantify root longevity, will be possible.

A review of published reports does not provide convincing evidence that elevated CO2 will either increase or decrease root turnover (Pritchard & Rogers 2000). From a theoretical perspective, it has been hypothesized that root turnover could either increase, remain the same, or even decrease in a higher CO₂ world (Van Noordwijk et al. 1998; Eissenstat et al. 2000; Pritchard & Rogers 2000). Tingey et al. (2000) reviewed the literature on root response of conifers to elevated CO₂ and found that root turnover responses ranged from an increase to a decrease. In contrast, root turnover has been reported to increase in a grassland (Fitter et al. 1997), Populus euramericana (Pregitzer et al. 1995), Betula papyrifera (Berntson & Bazzaz 1997), Liriodendron tulipifera (Norby et al. 1992) and Pinus radiata (Thomas et al. 1999) grown with high CO₂. Canadell et al. 1996) summarized several studies in which root turnover was measured. In four experiments with grass systems, increased root turnover occurred in three, while in the fourth there was no change. In four experiments on trees, increased turnover was observed in three, while the fourth showed a decrease.

Matamala & Schlesinger (2000) used sequential soil cores (bimonthly from June 1997 to November 1998) to quantify root increment, mortality and RNPP in this experiment. Their report serves as a benchmark to which other studies conducted at the Duke FACE site, utilizing different methods, can be compared (Matamala & Schlesinger 2000). They reported 46% and 68% increases in root mortality and production (RNPP), respectively, in elevated compared to ambient CO2 plots. These values differ substantially from our study (October 1998 to October 1999) in which increases of 46% and 26% were observed for mortality and production. Matamala & Schlesinger (2000) estimated root mortality (including decomposition) as 37.0 and 54.2 g dry wt m⁻² year⁻¹ under ambient and elevated CO2, respectively. These values are nearly identical to estimates of root mortality we obtained using the minirhizotron technique (34.7 and 50.8 g dry wt m⁻² year⁻¹, respectively). Values for RNPP

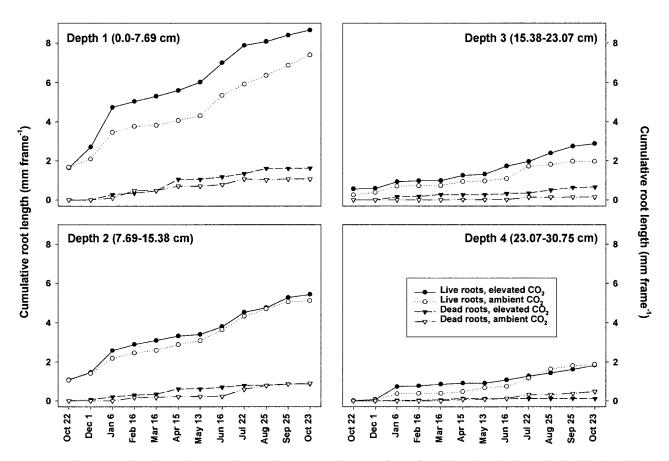


Fig. 3 Cumulative root length production and mortality per minirhizotron frame for all four depth classes for both high and low CO_2 availabilities.

(i.e. production), however, were substantially higher in our study (181.1 and 228.6 g dry wt m⁻² year⁻¹ in ambient compared to elevated plots) than those obtained through sequential soil coring (79.8 and 134.2 g dry wt m⁻² year⁻¹). Results obtained with both methods fall in the lower range for temperate and boreal forests. In fact,

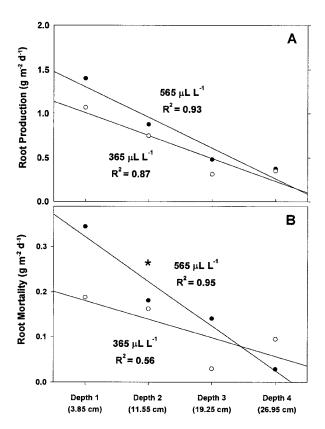


Fig. 4 CO₂ by depth interactions for (A) root production per day (not significant) and (B) mortality per day (P = 0.03). Productivity estimates were derived according to Tingey et al. (2000) using specific root lengths for this experiment reported by Matamala & Schlesinger (2000) (see text for details). Values are averaged over all dates. * indicates that the slopes of the regression lines differed at P < 0.10 as determined with a simple *t*-test.

the reported range of annual fine root production for pine forests is from 69 to 1090 g m⁻² year⁻¹ (mean of 331).

In the current study, all roots growing along the minirhizotron were included in the analyses whereas in the previous soil coring study, only roots that were < 1.0 mm were considered. This may partially explain why our mortality values are nearly identical (typically the finest roots are turned over more rapidly) while our productivity numbers, which include all roots, were higher. Alternatively, root dynamics over the period of September 1997 to May 1998 may have simply differed compared to the period from October 1998 to October

It should be noted that in our study, minirhizotrons had only been in place for 4 months prior to initiation of image collection. This may have allowed insufficient time for roots to colonize the tube surface and equilibrate with bulk soil following the disturbance associated with tube installation. Furthermore, there is some evidence that roots tend to grow preferentially along the tube-soil interface which may have elevated our estimates of RNPP. But, regardless of inaccuracies inherent with either method, similar qualitative patterns of root dynamics were observed using both sequential soil coring and minirhizotrons (Matamala & Schlesinger 2000).

Fairly consistent depth-by-CO₂ interactions along with regression analysis revealed that the stimulation of rooting by CO₂-enrichment in this loblolly pine forest was largely confined to the shallowest soil depths. Many other studies also report disproportionate stimulation of rooting at shallow depths (Pritchard & Rogers 2000). In a calcareous grassland in Switzerland, for example, Arnone et al. (2000) used minirhizotrons to determine that exposure to elevated CO₂ resulted in a shift in the vertical distribution of roots, with more being found in the upper layer of soil in the high CO₂ plots. Stimulation of root growth by high CO2 at shallow depths can sometimes be explained by greater soil water availability, enhanced nutrient availability as a result of nutrient mineralization in the litter layer (litter fall has been

Table 3 Estimated annual root production and mortality derived from minirhizotrons from October 1998 to October 1999. Values are means of 3 replicates (each with 12 minirhizotrons) averaged over all depths and dates

	Elevated	Ambient	F value	<i>P</i> > <i>F</i>
RNPP* (g dry wt m ⁻² year ⁻¹)	228.6	181.1	2.79	0.2366
Mortality (g dry wt m ⁻² year ⁻¹)	50.8	34.7	3.54	0.2006

^{*}Production and mortality were estimated by using a depth of field of 2.5 mm to convert from root length per minirhizotron frame (2 dimensions) to root biomass in bulk soil (3 dimensions) according to Tingey et al. (2000). Specific root lengths were derived from Matamala & Schlesinger (2000) to calculate root dry weights from root length numbers.

significantly increased in this experiment; Allen *et al.* 2000), or as a result of soil physical properties.

Our results suggest small, if any, increases in root proliferation (and then mostly at shallow soil depths). These modest but insignificant increases in rooting, considered along with previous data of Matamala & Schlesinger (2000), do suggest, however, that rooting will be enhanced to some extent and that nutrient acquisition within loblolly pine forests may increase as atmospheric $[CO_2]$ rises. In this loblolly pine forest, the non-significant (P=0.23) enhancement of root productivity (+ 26%) is nearly identical to the increase in above-ground NPP (+ 25%) observed in the first 3 years (DeLucia *et al.* 1999) suggesting that, at the ecosystem level, allometry of C allocation to root growth vs. shoot growth is relatively stable at elevated levels of atmospheric CO_2 .

Enhanced root production and mortality could potentially lead to sequestration of atmospheric CO₂ in soil organic C, thus partially ameliorating further increases in atmospheric CO₂. However, on the other hand, faster turnover times of short-lived tissues such as leaves and fine roots could constrain the size of this carbon sink. Based on the observation that carbon accumulation in deeper soil mineral layers of CO₂-enriched plots was absent in this experiment, Schlesinger & Lichter (2001) recently questioned the potential of soils for long-term carbon sequestration. Clearly, the issue concerning the contribution of fine roots to long-term carbon sequestration in forest soils remains to be resolved (Davidson & Hirsch 2001).

The lack of significant differences observed in the current study, often in spite of large percentage differences, indicates that it will be very difficult to quantify ecosystem-level root dynamics with minirhizotrons alone. Here, experimental rings were replicated only three times, and although 12 separate minirhizotron tubes were installed into each experimental ring as subsamples, high variability still precluded detection of statistically significant differences. Without greater replication (which may be unpractical considering the expense associated with maintaining each experimental ring), minirhizotrons may be most useful when used in combination with other methods, such as sequential soil coring. In fact, in natural settings (the goal of FACE studies), employing multiple methods may prove to be the only approach that will result in meaningful understanding of root dynamics and below-ground ecosystem functioning, regardless of replication number.

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